Embryoid Bodies and the Ambr®15: Improving Expansion of Pluripotent Stem Cells in Stirred Tank Reactors

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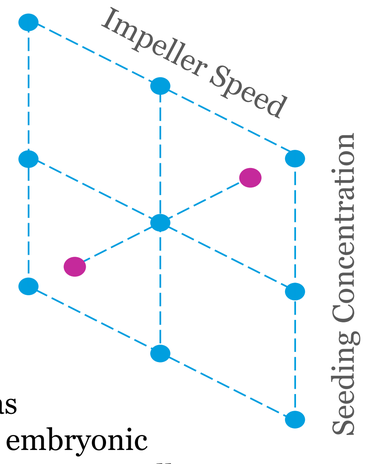
Aims

The use of 2D technologies for the controlled expansion and differentiation of pluripotent stem cells (PSCs) is cost limiting when scaled to manufacture allogeneic therapies. The Cell and Gene Therapy Catapult (CGT) is developing bioprocesses for cost-efficient expansion of PSCs in high-density, embryoid body (EB) suspension culture, employing Quality-by-Design methodologies. We have applied the Ambr®15 micro-bioreactor system to investigate the effects of impeller speed, seeding concentration, and feeding regime on the expansion of two PSC lines.

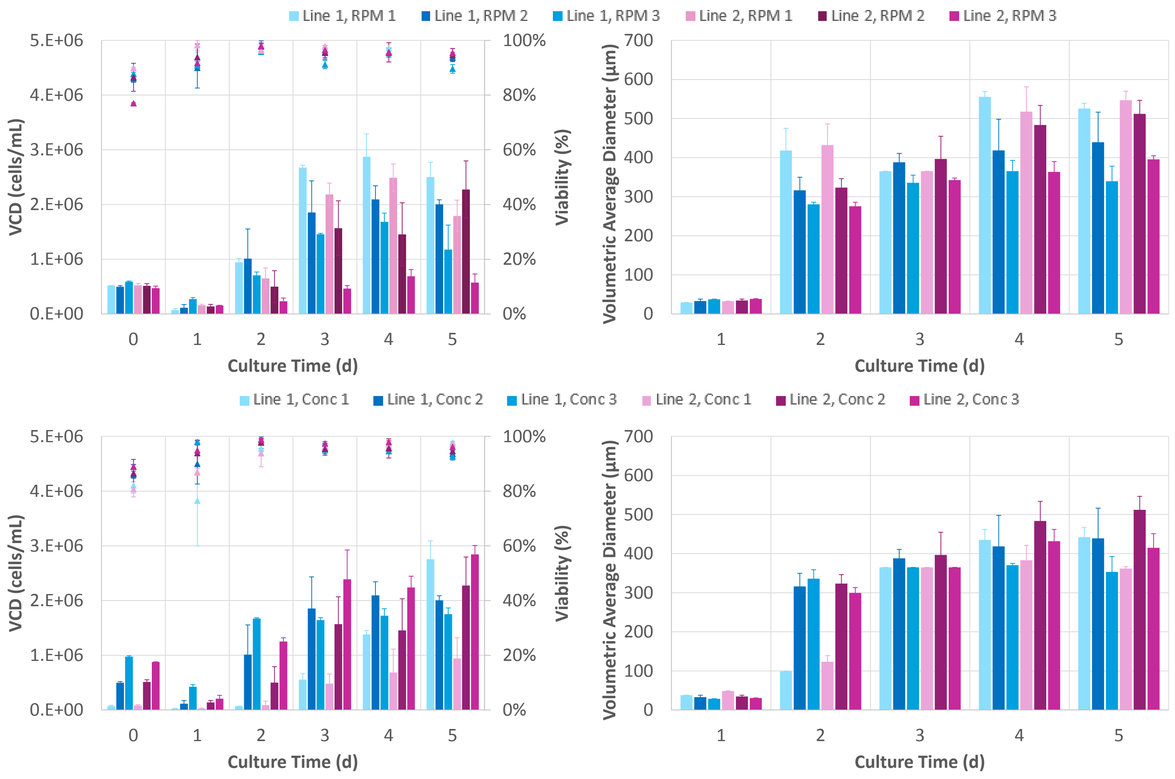
Design

A range of impeller speeds and seeding concentrations were explored using a face centred central composite design. An initial screen of feeding regimes was conducted at the mid-point values. In addition to the standard fixed-volume medium exchange, we screened a higher frequency fixed-volume exchange and a higher frequency exchange with a volume dependent on cell concentration.

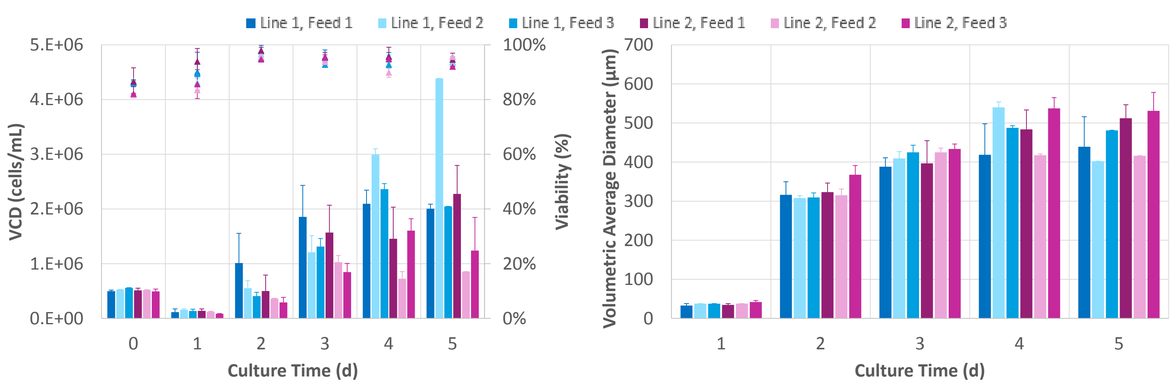


Shef6.1 (Line 1) and RCiB10 (Line 2) cell lines were used as exemplars of human embryonic and induced pluripotent stem cells, respectively. All Ambr®15 vessels were seeded with single-cell suspension and utilised a rho-kinase inhibitor for the first 24 hours. Cells were harvested after 5 days of culture. All conditions were screened in duplicate (n=2) except the centre point conditions (n=4).

Growth, Viability and Size

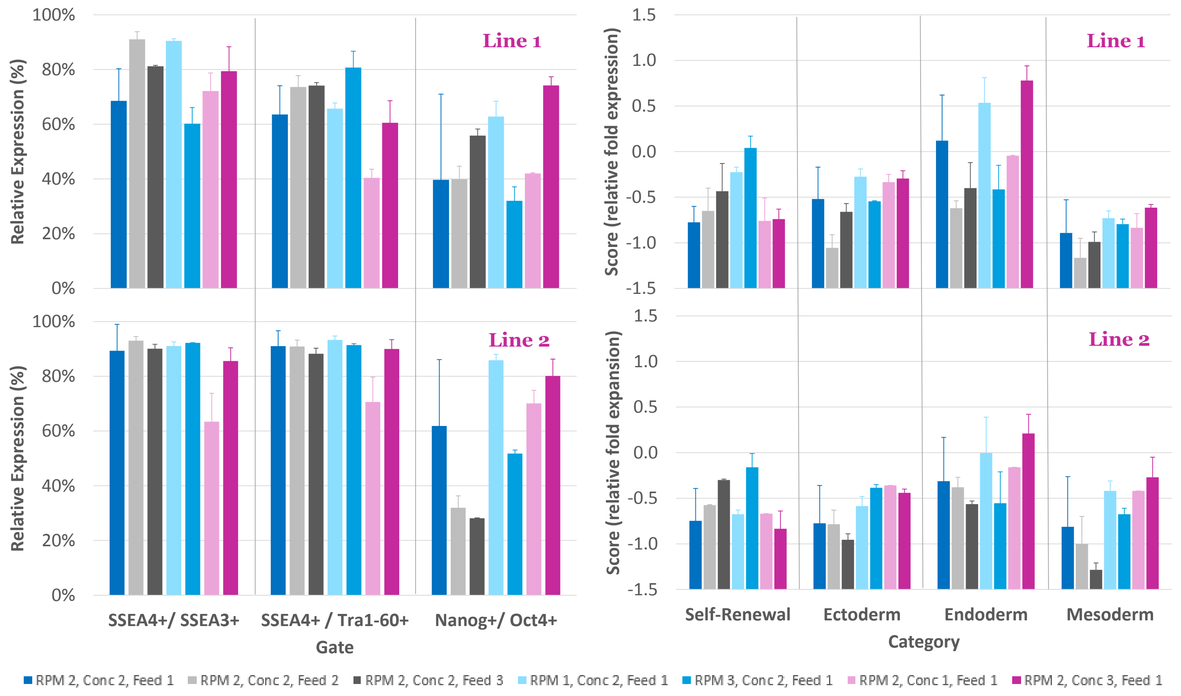


Cell counts and viability were measured after dissociation of EBs. Viability (triangles) follows the same trend in all samples with the lowest viability occurring at the day of seeding. EB diameters were analysed from microscope images, the average EB volume calculated, and the corresponding diameter plotted. Higher impeller speeds (top) show a positive effect on day 1 cell survival but a negative effect on total population doublings. Higher impeller speeds also result in larger EBs on day 1 but smaller EBs on subsequent days. Higher seeding concentration (bottom) had a negative effect on the final cell density of cell line 1 but a positive effect on cell line 2.



Relative to daily, fixed-volume medium exchange (Feed 1), twice-daily, fixed-volume medium exchange (Feed 2) significantly increased the expansion of cell line 1 during the later days of culture. Twice-daily medium exchange with a volume based on cell count (Feed 3) did not have a similar effect, despite similar exchange volumes over the final day of culture. Cell line 2 showed the highest expansion with daily, fixed-volume medium exchange.

Pluripotency

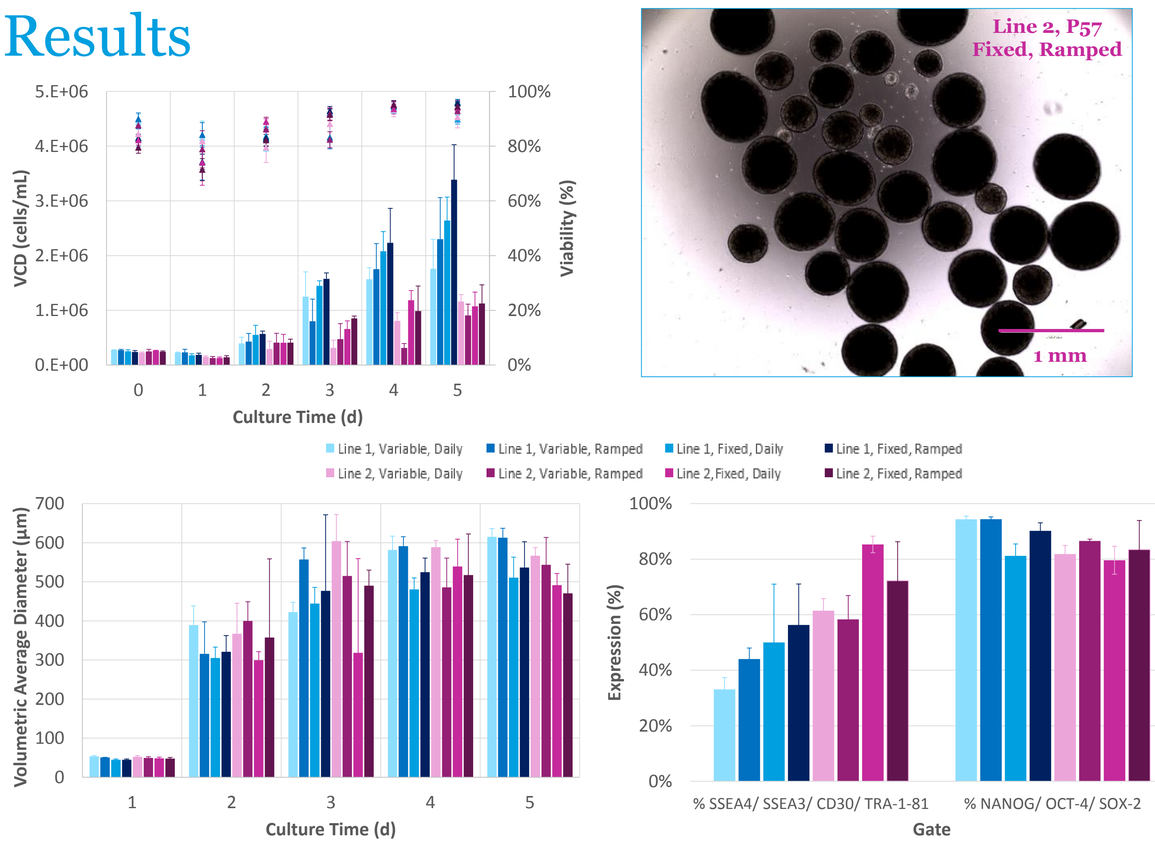


FACS gates show the percentage of live, single cells, stained positively for each pair of pluripotency markers. Scores are the output of the TaqMan® hPSC Scorecard™ assay. FACS data suggests that increasing seeding concentration has a positive effect on pluripotency but this is contradicted by a higher relative score observed for the expression of genes in the mesoderm and endoderm categories. Higher impeller speeds show a positive effect on self-renewal, and a negative effect on germ layer scores and Nanog/Oct-4 expression. These trends may relate to the lower EB sizes observed.

Secondary Design

A secondary experiment was designed to further investigate observed trends. Fixed and varied impeller speeds were investigated in combination with fixed and ramped medium exchange frequencies. The same cell lines were utilised and each combination of conditions was investigated in triplicate (n=3).

Results



Higher day 1 cell survival was confirmed for higher impeller speeds (variable). However, a reduction in impeller speed from 24 hours did not cause the higher cell numbers to persist to day 2. The two feeding regimes did not diverge until 72 hours. From day 3, a clear increase was observed in the rate of expansion of cell line 1 with increasing medium exchange frequency (ramped), relative to daily exchange. This is consistent with expectations from the previous investigation. Similarly, increased medium exchange frequency did not increase the rate of expansion of cell line 2. An expanded FACS panel was used for the secondary design with the percentage of live, single cells that stain positively for all surface and intracellular pluripotency markers shown. Fixed impeller speeds resulted in higher expression with both lines.

Relevance and Ongoing Work

The results provide an operational design space of impeller speed, seeding concentration, and feeding regime for further exploration in larger scale expansion.

CGT are currently working to determine appropriate scaling factors for scale-up to larger STR systems, and engaged in the design and assessment of cell retention technologies to enable process intensification.

We work with Innovate UK

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